

Claims 2-5 and 12-18 have been cancelled and are replaced with new claims 22-45. Claims 22-53 are pending in the case.

The Invention

The invention provides novel microclonal cDNA libraries, each representing collections of gene transcripts expressed by the progeny of a single stem/progenitor cell that proliferates to provide a microclonal population. As described in the specification §§4.5 and 4.6, microclones can be created from a variety of stem cells or progenitor cells derived from diverse tissues including bone marrow, tumors and brain tissue.

Microclones derived from brain stem/progenitor cells are also known as "neurospheres."

Work by Applicants (Kukekov et al., 1999) has shown that individual brain cell microclones are composed of a diverse population of differentiating neural morphotypes, leading to the recognition of the microclone as an "isolated miniature model of neurogenesis." Furthermore, Applicants have shown using anatomical and molecular biological techniques that brain cell microclones are heterogeneous with respect to one another. This heterogeneity is believed to be due to the state of differentiation of the initial stem or progenitor cell that gave rise to the microclone. Accordingly, the collection of gene transcripts expressed in the progeny cells of any given neurosphere is determined by the pluripotential or multipotential capability of the initial stem or progenitor cell that gave rise to the microclone.

The present invention provides microclonal cDNA libraries and gene arrays derived from individual microclones. The invention takes advantage of the heterogeneity that exists among microclones to isolate and thereby allow identification of gene transcripts that are expressed by different microclones at progressive points along the sequence of development from a primitive, asymmetrically dividing pluripotential stem cell to a fully differentiated cell type. As described in the specification on p. 12, lines 26-28, brain cell microclones representing early stages of neurogenesis yield cDNA libraries containing genes expressed during early neurogenesis whereas those representing late stages of neurogenesis yield cDNA libraries containing genes expressed during late neurogenesis.

Applicants prepared microclonal cDNA libraries from microclones derived from tumor cells (see Example 6) and from brain neurospheres at different stages of maturity, as shown in FIG. 2 of the application. The stage of development of the neurospheres was determined by analyzing each library with a panel of known marker genes associated with different stages of neural development. Based on the subset of markers expressed in each microclonal library, the cDNA libraries could be arranged by use of an algorithm in a sequential order according to the presence of specific markers reflecting emergence of progressively more differentiated cell types. See Table 4, p. 45 of the specification. Subtractive cDNA libraries could then be prepared from adjacent microclonal libraries in the sequence, to reveal further markers expressed at specific stages of development.

Rejections Of Claims 2-5 And 12-18 Under 35 U.S.C. §101

Claims 2-5 and 12-18 were rejected under 35 U.S.C. §101 as directed to non-statutory subject matter. Claims 2-5 and 12-18 having been cancelled, this rejection is rendered moot. Applicants respectfully contend that new claims 22-45 constitute statutory subject matter.

Rejections of Claims 2-5 And 12-21 Under 35 U.S.C. §102 (a)

Claims 2-5 and 12-21 were rejected under 35 U.S.C. §102 (a) as anticipated by PCT application WO 99/11758 to Cytotherapeutics, Inc. ("Cytotherapeutics"); by Adams et al. (Nature Genetics 4:373-380, 1993); and by Arsenijevic et al. (J. Neurosci. 18:2118-2128, 1998).

Claims 2-5 and 12-21 having been cancelled by this amendment, these rejections are moot with respect to these claims. New claims 22-46 relate to a "microclonal cDNA library," "a subtractive cDNA library," and a "DNA array." Applicants respectfully submit that the new claims are not anticipated by any of the references used in the rejection of former claims 2-5 and 12-21 for the following reasons.

In Cytotherapeutics, the term "cDNA library" is used only once, i.e. in claim 19. Aside from this one reference, the international application is devoid of any mention whatsoever of cDNA libraries. The claimed cDNA library in Cytotherapeutics refers to cultures comprising human neural stem cells. This reference contains no written

description of how one of skill would make *any* cDNA library from the cultures of Cytotherapeutics. Furthermore, Cytotherapeutics does not disclose how to make and use any of Applicants' claimed microclonal libraries, subtractive cDNA libraries or DNA arrays. At the most, Cytotherapeutics discloses cDNA libraries prepared from *whole cultures* containing neural stem cells. Such cultures are known to contain *populations* of neurospheres, each at a different stage of development. Thus a cDNA library prepared from such cultures contains a mixture of all of the transcripts expressed by neurospheres at every stage of development present in the culture. Temporally related transcripts are not separated from one another in such a cDNA library. For this reason, such cDNA libraries are not useful for characterizing subsets of gene expressed during a developmental sequence such as neurogenesis. Therefore the Cytotherapeutics reference does not anticipate Applicants' invention.

As discussed above, Applicants' invention allows for isolation and characterization of genes expressed during a developmental sequence precisely because the microclonal cDNA libraries and the subtractive cDNA libraries are restricted to a subset of transcripts expressed *within a single microclone*, captured at a precise stage in its development. A great advantage of these isolated subsets of genes is their use in correlating gene expression with stages in developmental sequences such as neurogenesis, hematopoiesis and oncogenesis, and in facilitating discovery of new genes expressed in relation to specific events during these developmental processes. Such information cannot be derived from a cDNA library containing a random mixture of all of the transcripts expressed by cultures containing mixed populations of microclones, each at a different stage of development.

For similar reasons, Applicants' invention is not anticipated by Adams et al. The Adams reference describes the preparation of a cDNA library from a 73-day old human infant brain. Having been generated by standard methods of cDNA library construction from total RNA extracted from this brain, this library provides a "snapshot" of all transcripts expressed in this brain on day 73 of life. Separate microclones were not prepared, and gene markers for distinct stages of development were neither analyzed nor correlated with expression of the markers during neuromorphogenesis. As explained

above, such analyses are not possible using a library containing the totality of transcripts expressed by a tissue at a single point in time.

Claims 2-5 and 12-21 were also rejected as anticipated by Arsenijevic et al. The Action notes that Arsenijevic discloses isolation of total RNA from neurospheres comprising stem and progenitor cells. The Action appears to suggest that Arsenijevic is describing a sequential neurogenesis profile. The Arsenijevic reference neither teaches nor suggests characterizing different isolated clones (neurospheres) at different times of development and then arranging the clones in relation to stage of maturation. It may be illustrative to refer to the overall objective stated in the Arsenijevic reference: to determine "whether IGF-1 could promote the in vitro differentiation of post-mitotic mammalian CNS neuronal precursors derived from multipotent epidermal growth factor (EGF)-responsive stem cells" (page 2118, second sentence of the Abstract). On page 2119 (right col, 3rd full paragraph), reverse transcriptase and PCR methods for isolating and determining the presence of IGF-II receptor are described. Total RNA was not isolated from individual neurospheres, and cDNA libraries were neither constructed nor mentioned in the reference.

For the reasons stated above, Applicants submit that the invention was not anticipated by any of the cited references and request withdrawal of the rejections.

Rejections Of Claims Under 35 U.S.C. §112, ¶1

Claims 2-5 and 12-21 were rejected under 35 U.S.C. §112, ¶1, for purportedly claiming subject matter not adequately described to convey to one of skill in the art that Applicants had possession of the invention at the time the application was filed. Claims 2-5 and 12-21 having been cancelled by this amendment, these rejections are rendered moot.


Rejections Of Claims Under 35 U.S.C. §112, ¶2

Claims 2-5 and 12-21 were rejected under 35 U.S.C. §112, ¶2. Claims 2-5 and 12-21 having been cancelled by this amendment, these rejections are moot.

Applicants intend this paper to be fully responsive to the issues raised in the office action and respectfully request reconsideration of the application. No new matter has

been added. Should the examiner have any questions or suggestions, the undersigned requests a telephone conference.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Gregory A. Nelson', written over a horizontal line.

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